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Impact of Genomic Variation of NFE2L2 (rs6706649) on Serum Glyoxalase-1 Levels in A Sample of Iraqi Type 2 Diabetic Patients with Retinopathy

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Abstract

Nuclear factor erythroid-2-related factor-2 (NFE2L-2) is one important endogenous anti -oxidative stress signal pathway. Single nucleotide polymorphism (SNP) or genetic variants of NFE2L-2 may be accountable for the genesis of diabetic retinopathy. Glyoxalase-1 (GLO-1) is the rate-limiting enzyme in the detoxification of methylglyoxal (MG) into D-lactate. The activity of GLO-1 is regulated by the transcription factor NFE2L2**.**The goal of this study is to ascertain whether there is an association between the rs6706649 of NFE2L2 gene variant with serum GLO-1 levels in a group of Iraqi diabetic patients diagnosed with diabetic retinopathy (DR). This study included 90 patients diagnosed with type 2 diabetes mellitus (48 females and 42 males), with ages ranging from 40 to 80 years. The participants were partitioned into two the following groups: **Group A**; 60 patients with type 2 diabetes mellitus diagnosed with DR (among them 29 patients with non-proliferative diabetic retinopathy (NPDR) and 31 patients with proliferative diabetic retinopathy (PDR)) and **Group B:** 30 patients without evidence of DR (DWR) considered as a control group were enrolled in the study. A non-significant difference in genotyping and allele carriage frequencies of rs6706649 (G/A) SNP between DR and DWR groups. Also, there was a non-significant difference between PDR and NPDR patients' groups in relation to the different genotypes and alleles of rs6706649 (G/A) of NFE2L2 gene. However, the changing from the wild genotype (GG) to mutant (AA) and heretogynotypes (GA) had significant positive correlation with the increased serum levels of GLO-1 and a significant negative correlation with the serum level of methylglyoxal (MG) in patients with diabetic retinopathy. Furthermore, pentosidine, carboxymethyl lysine (CML), GLO-1 and MG levels were significantly higher in DR when compared to DWR groups. The A mutant allele of rs6706649 (G >A) had a significant positive correlation with the increased serum GLO-1 levels in DR group. Meanwhile, DR group had increased serum MG, GLO-1, pentosidine and CML levels. Hence, these biomarkers can serve as prognostic indicators for diabetic retinopathy.

Keywords: Oxidative stress, NFE2L-2 gene polymorphism, GLO-1, MG, diabetic retinopathy.

Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder that affect many people over time [(1)]. Diabetic retinopathy (DR) is a gradual and chronic neurovascular consequence of diabetes mellitus (DM). Irreversible vision deterioration is frequently caused by this prevalent condition [(2)]. There are two phases of DR include a non-proliferative (NPDR) and proliferative (PDR) [(3)]. The development of advanced glycation end products (AGE) is directly related to the regulation of blood glucose levels. They change the structure and function of blood vessels and interact with receptors for AGEs, triggering intracellular signaling those results in heightened oxidative stress and the release of important pro-inflammatory cytokines [(4,5)]. The accumulation of AGEs in retinal cells is strongly associated with the development of DR [(6)]. Additionally, reactive dicarbonyls are produced during glucose metabolism , but there are particular protective processes that may detoxify them. These safeguards reduce AGE buildup in tissues by clearing AGEs intermediates, since most AGEs are irreversible once generated [(7)]. The glyoxalase system is the principal detoxifying mechanism for these reactive dicarbonyls. D-lactate is the end product of the glyoxalase system, and it is substantially less reactive than methylglyoxal (MG), which is far more reactive. These reactions are carried out by the enzymes glyoxalase-1 (GLO-1) and glyoxalase-2 (GLO-2), and they require a catalytic amount of GSH [(7)]. Among the AGE, pentosidine and carboxy-methyl-lysine (CML) are produced as a result of subsequent reactions. Accumulated AGEs enhance ROS generation,

leading to an increase in AGE production. The prevalence of these AGEs is strongly associated with the severity of DR [8]. Serum pentosidine and CML levels in patients with type 2 diabetes are correlated with the degree of retinopathy [9]. Oxidative stress is significantly controlled by the nuclear factor erythroid-2-related factor -2 (NFE2L2), which controls the antioxidant response element (ARE)-mediated transcription of antioxidant enzymes [4]. Some genetic variations, particularly single nucleotide polymorphisms (SNPs), can contribute to the development of diabetic complication [10]. Disease susceptibility variants are related to variations in the induction of nucleotide substitution at specific sites in genes [11]. Researchers have shown that some variations in the NFE2L2 promoter region are associated with oxidative stress-related disorders. This suggests that these variations may have a hereditary role in determining disease susceptibility [12] Furthermore, ARE is located in GLO-1 exon 1, suggesting that NFE2L2 affects GLO-1 activity. GLO-1 can be induced by NFE2L2 activators such as sulforaphane and resveratrol [13]. The objective of this research is to determine whether there is an association between the genetic variations rs6706649 G/A in the promoter region of the NFE2L2 gene with serum GLO-1 levels in a group of Iraqi patients diagnosed with DR.

Materials and Methods

Patients

An observational case-control study was conducted on a cohort of Iraqi individuals with type 2 diabetes mellitus (T2DM), who had been diagnosed with the T2DM for at least 5 years ago. The participants in this study were chosen from the individuals seeking medical care at Ibn Al-Haitham Hospital of Ophthalmology and the Specialized Center for Endocrinology and Diabetes in Baghdad, Iraq. The recruitment commenced in February 2023 and terminated in July 2023. The research protocol has been approved by the College of Pharmacy Scientific and Ethical Committee, University of Baghdad (REAFUBCP3112023A), besides an informed consent was obtained from each participant in the study. All the participants were interviewed by the researcher and demographic data were obtained from them and recorded in a data collecting sheet, including age, gender, duration of disease, body weight and height, educational level, past medical history and drugs used in treatment of DM. A total number of 102 participants initially participated in the study. Nevertheless, the blood samples from twelve patients were omitted from the study because of their hemolysis. The remaining 90 patients were categorized into: **Group A** consisted of sixty individuals who were diagnosed with type 2 diabetes and retinopathy. Their ages ranged from 40 to 80 years old. Within this group, twenty-nine patients had non-proliferative diabetic retinopathy (NPDR) and thirty-one patients had proliferative diabetic retinopathy (PDR). The diagnosis of retinopathy was made by an ophthalmologist using the early treatment diabetic retinopathy study (ETDRS) criteria, with the assistance of optical coherence tomography (OCT) to identify the presence and location of intra-retinal and/or sub-retinal fluid, as well as retinal hemorrhages and microaneurysms [14]. **Group B** consisted of thirty type 2 diabetes patients without retinopathy, serving as the control group.

Inclusion criteria

Patients were selected to be previously diagnosed with type 2 diabetes mellitus according to American diabetic association (ADA) diagnostic criteria [15]. The diabetic patients must be within age between (40-80) years. The Duration of diabetes mellitus in participant patients must be greater than five years.

Exclusion criteria

- Patients whom have Type 1, gestational diabetes mellitus and Type 2 diabetic patients on insulin therapy, were excluded.
- Diabetic patients with cardiovascular, liver and renal diseases, acute bacterial and viral infection, autoimmune diseases and ocular diseases, nor the diabetics using multivitamin supplements, nor those with diabetic retinopathy on antiVEGF drugs were excluded.

Specimen collection and handling

From each participant, ten milliliters (ml) of venous blood were drawn by venipuncture. Five ml of the obtained blood sample was transferred into two ethylene diamine tetra acetic acid (EDTA) tubes; into the first tube, two ml of blood was poured to be used for HbA1c assay by modified enzymatic reagent for the in vitro determination of HbA1C in human blood, whereas the second tube was filled with three ml for DNA extraction and analysis. The first EDTA tubes were taken directly to the laboratory for HbA1c assay. The remaining second EDTA tube was frozen at (-20 °C) until the time of DNA extraction. Five ml of the remaining whole blood was transferred to a gel tube and allowed to coagulate for 30 minutes before being centrifuged for 10 minutes at 3000 rounds per minute (rpm) to get the serum. On the day of collection, a portion of the serum was used by the hospital's laboratory to measure fasting serum glucose (FSG) by an enzymatic colorimetric method. The residual serum was kept as aliquots in Eppendorf tubes and kept frozen at (–20°C) until complete sample collection. Following that, MG, GLO-1, Pentosidine and CML were quantified using specific enzyme-linked immunosorbent assay (ELISA) kits.

Genotyping

Genomic DNA was extracted from the peripheral blood leukocytes (frozen EDTA blood samples) by Easy Pure Blood Genomic DNA Kit and the concentration of DNA samples that showed an acceptable integrity were estimated by using a Nanodrop spectrophotometer (Thermo Fisher Scientific). Geneous prime was used to design PCR primers (Table 1). They were synthesized and lyophilized by Alpha DNA Ltd. (Canada). The genotyping reaction in PCR was performed by a thermal cycler device (QIAamplifier96- QIAGEN, Germany) using a master mix from TransGen biotech, China.

Table 1. Primers

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Annealing Temperature (°C)
rs6706649 – F	TCGTTGATTCCACAGCATTT	20	675	54
rs6706649 – R	GAGTTCGGACGCTTTGAAAC	20		

rs6706649 -F: Forward primer, **rs6706649 -R**: Reverse primer, **bp**: base pair

Primer optimization

To determine the optimal annealing temperature for primers, the pair of primers (Forward) (Reverse) was used to amplify the template of DNA at (54, 56, 58°C) annealing temperatures. The best annealing temperature for the primer was 54°C for producing clear and sharp bands in agarose gel, hence it was used in the current study. Sanger's technique was used for DNA sequencing for the purpose of determining the presence or absence of SNPs within the primed amplified region of the NFE2L2 promotor region.

Statistical analysis

Statistical analysis of data was performed using IBM SPSS. Continuous variables were expressed as mean ± SD of the values. The mean differences of two independent samples were measured by unpaired t-test, while one-way analysis of variance (ANOVA) was utilized for more than two groups. A post-hoc analysis was performed. Fisher's exact or Chi-square tests were utilized to measure the group differences between categorical variables. Binary logistic regression analysis and Pearson correlation was performed, A probability that equals or less than (0.05) indicates a significant difference.

Results

Demographics Data between two groups of type 2 diabetic patients with retinopathy and diabetic patients without retinopathy

By the measurement of p-value for the mean values of PDR, NPDR, and DWR among study groups were demonstrated statistically significant differences for age, duration of diabetics, and smoking between these groups, whereas the mean values of BMI and gender exhibited non-significant differences between them in as presented in the (Table 2).

Table 2. Demographic data for type 2 diabetic patients with two stages of retinopathy (PDR And NPDR) and diabetic patients without retinopathy

Characteristics		NPDR (N=29)	PDR (N=31)	DWR (N=30)	P-value
Age (years) Mean ± SD		57.59±8.382 [a]	56.23±8.601[a]	51.77±8.529 [b]	0.02*
BMI Mean ± SD		29.9847±5.15348	28.0697±3.06455	29.4913±4.94645	0.2
Duration of Diabetes (years) Mean ± SD		13.48±5.865 [a]	13.23±5.290 [a]	9.60 ±6.333 [b]	0.01*
Gender N (%)	Male	15 (51.7%	17 (54.8%)	10 (33.3 %)	0.1
	Female	14 (48.3%)	14 (45.2%)	20 (66.7 %)	
Smoking N (%)	Yes	4(13.8%)	8 (25.8%)	1 (3.3%)	0.04*
	No	25 (86.2%)	23 (74.2%)	29 (96.7%)	

Means followed by different letters like(ab) are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letters are not significantly different. DWR: diabetic without retinopathy, NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, N: number, BMI: body mass index, SD: standard deviation, *: significance difference ($P<0.05$)

Biochemical characteristics of diabetics with and without retinopathy

The pentosidine, CML, GLO-1 and MG were statistically highly significant differences in PDR and NPDR when compared to DWR groups. Data presented in the (Table 3) showed that the serum levels of pentosidine, CML, GLO-1 and MG were higher in PDR and NPDR than those in DWR groups, but there were no significant differences between PDR and NPDR. Although, the PDR groups had elevated FSG levels compared to NPDR and DWR groups but, there were no significant differences among the three groups. The results of HbA1c data showed significantly higher levels in PDR group than those in NPDR and DWR groups. Conversely, HbA1c levels demonstrated no significant differences between NPDR and DWR groups. (Table 3)

Table 3. Biochemical characteristics of diabetic patients with two stages of retinopathy and without retinopathy

Marker	PDR (N=31) Mean ± SD	NPDR (N=29) Mean ± SD	DWR (N=30) Mean ± SD	P-value
FSG mg/dl	221.61±71.307	193.07±72.896	204.90±72.844	0.3
HbA1C	9.69±1.724 [a]	8.23±1.733 [b]	8.361.736 [b]	0.002**
MG (ng/ml)	24.030 ± 8.098[a]	24.770 ± 6.752[a]	9.792 ± 1.957[b]	0.0001**
GLO-1 (ng/ml)	34.349 ± 5.697 [a]	32.051 ± 6.182 [a]	16.708 ± 4.319[b]	0.0001*
Pentosidine (pmol/ml)	686.992±180.246[a]	672.330 ±202.122[a]	265.027±74.803 [b]	0.0001**
CML (ng/ml)	2164.571 ±296.351[a]	2094.039±287.420[a]	1045.839±237.0[b]	0.0001*

Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letters are not significantly different. NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, DWR: diabetic without retinopathy, N: number, SD: standard deviation, N: number, CML: Carboxymethyl lysine, GLO-1: Glyoxalase-1, MG: Methylglyoxal, FSG: Fasting serum glucose, pmol: Picomoles, ng: nanogram , mg: milligram, dl: deciliters, ml: milliliter, **: highly significant difference (P<0.01).***: very highly significant difference (P<0.001).

Correlations between parameters and biomarkers included in the current study

The results illustrated in (Table 4) shows the Pearson's correlation analysis for the association between the serum biomarkers levels. A significant positive association was found among serum levels of pentosidine, GLO-1, MG and CML. The results of FSG levels were demonstrated no association with studied biomarkers while there was a positive correlation with HbA1C.

Table 4. Pearson's correlation analysis of the association between the serum biomarkers included in the study for type 2 diabetic patients with and without retinopathy

Variables		FSG	HbA1c	pentosidine	GLO1	CML	MG
FSG	*r*	1	0.515**	-0.103	0.031	0.063	-0.054
	p		0.0001	0.333	0.772	0.554	0.615
HbA1c	*r*		1	0.152	0.203	0.204	0.069
	p			0.154	0.055	0.054	0.515
pentosidine	*r*			1	0.677**	0.792**	0.646**
	p				0.0001	0.0001	0.0001
GLO-1	*r*				1	0.670**	0.722**
	p					0.0001	0.0001
CML	*r*					1	0.601**
	P						0.0001
MG	*r*						1
	P						

CML: Carboxymethyl lysine, GLO-1: Glyoxalase-1, MG: Methylglyoxal, FSG: Fasting serum glucose, ***: very highly significant difference(P<0.01)

Result of genetic analysis

The rs6706649 (G/ A) SNP in the NFE2L2 gene was analyzed by the Sanger method of sequencing (Figure.1). The finding of a single G peak represents [G] homozygous allele. In contrast, the presence of only A peak suggests a [A] homozygous allele. Moreover, both G and A peaks indicate the [G/A] heterozygous allele.

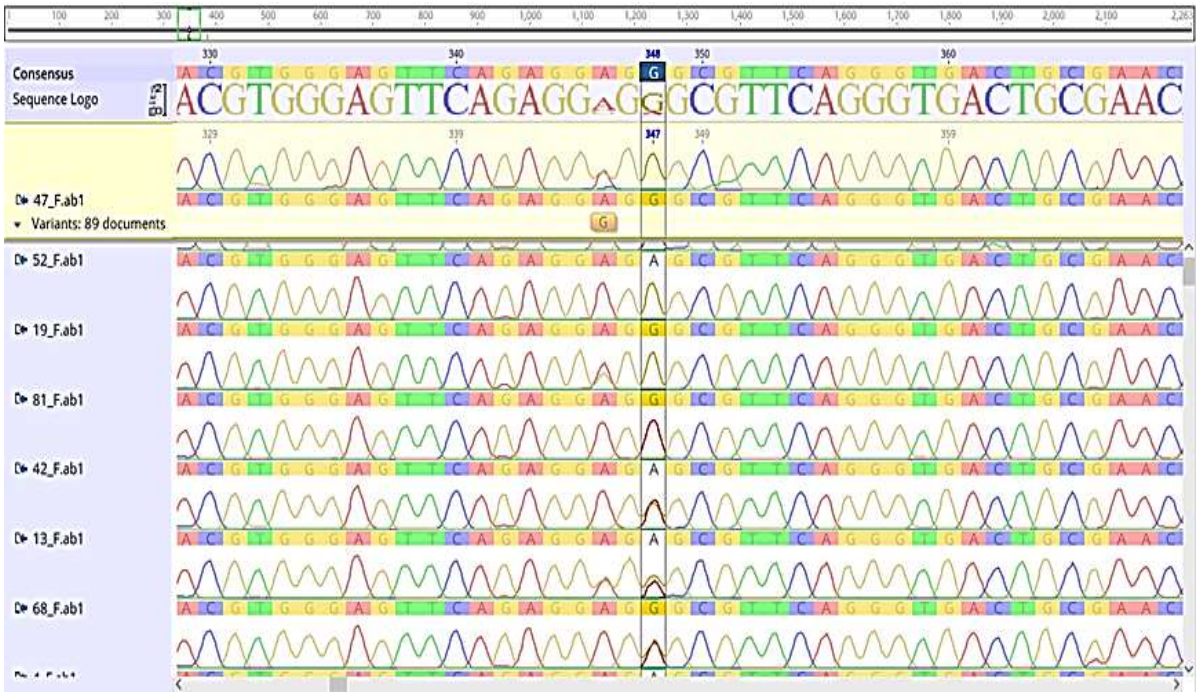

Figure 1. Analysis of rs6706649 (G/ A) SNP of the NEF2L2 gene

The prevalence of rs6706649 (G/A) SNP in all study participants

Prevalence of (rs6706649) G/A in the promoter region of NFE2L2 gene in all study participants. The results in the (Table 5) demonstrated that the GG (wild genotype) was the most prevalent in DR and DWR groups. Additionally, AA (mutant genotype) had a small incidence in the DR group and it was absent in the controls. Notably, the G and A allele had higher frequency in DR group than the DWR group. A non-significant difference in genotyping and allele carriage frequencies of rs6706649 G/A SNP between DR and DWR groups. The wild genotypes GG of rs6706649 of NFE2L2 gene in PDR group had higher frequency than those in NPDR group. However, there are non- significant differences in this genotype within the diabetic retinopathy patients' group. Additionally, the G allele was the most prevalent in PDR group enrolled in the study. There was a non-significant difference between PDR and NPDR patients' groups in relation to the different genotypes and alleles of rs6706649 of NFE2L2 gene (Table 6).

Table 5. Frequency of genotypes and alleles of rs35652124 in type 2 diabetic patients with and without retinopathy

SNP	Genotype	DR (N= 60)	DWR (N= 30)	P-value	Odd ratio (95% CI)
		N (%)	N (%)		
rs6706649	GG +	47 (78.3)	21 (70.0)	0.38	**1.00 (Reference)**
	GA	12 (20.0)	9 (30.0)	0.55	**0.59 (0.4345 to 2.97)**
	AA	1 (1.7)	0(0.0)	1	**1.3 (0.0531 to 34.70)**
	Allele				
	G	106 (88.33)	51 (85)	0.52	**1.00 (Reference)**
	A	14 (11.66)	9 (15)	0.52	**0.74 (0.2952 to 1.12)**

DR: diabetic retinopathy, DWR: diabetic without retinopathy, N: number, CI: Confidence interval

Table 6. Frequency of genotypes and alleles of rs6706649 in PDR and NPDR type 2 diabetic patients

SNPs	Genotype	PDR (N= 31) N (%)	NPDR (N= 29) N (%)	P -value	Odd ratio (95% CI)
rs6706649	GG+	25 (80.6%)	22 (75.9%)	0.65	1.00 (Reference)
	GA	6 (19.4%)	6 (20.7%)	0.89	0.8 (0.2475 to 3.1285)
	AA	0 (0.0%)	1 (3.4%)	0.48	0.29 (0.0114 to 7.5891)
	Allele				
	G	56 (90.3)	50 (86.2)	0.48	1.00 (Reference)
	A	6 (9.6)	8 (13.7)	0.48	0.6 (0.2174 to 2.0629)

NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, N: number, CI: Confidence interval, *: significance difference (P<0.05), +: The wild genotype

Correlations between genotypes and serum biomarkers in studied groups

Table 7 highlighted that a highly significant differences of the serum pentosidine, CML, GLO-1 and MG levels in wild and hetero genotypes of rs6706649 SNP of NFE2L2 gene between DR and DWR groups. The results in the (Table 7) revealed a significant difference in pentosidine between GG and GA genotypes of rs6706649 SNP in DWR group. The GA genotype of rs6706649 SNP of NFE2L2 gene had a statistically significant difference in FSG levels between PDR and NPDR groups while the HBA1C levels in the carriers of GG genotype were significantly differed between PDR and NPDR group. Post hoc analysis revealed a non-significant difference among the various genotypes (GG, GA, AA) within the DR patient's groups. However, the GG, GA, and AA genotypes in NPDR group were differed statistically in the serum of MG levels, Table 7.

Table 7. Serum levels of studied parameters with variant genotypes of NFE2L2 SNP (rs6706649) among PDR, NPDR and diabetic patients

Marker	Genotype	DR (n=60) M±SD		DWR (n=30) M±SD	P-value
		PDR (N=31)	NPDR (N=29)		
FSG	GG^+	211 ± 71	206 ± 78	212±80	0.9
	GA	264 ± 62[a]	155 ± 30[b]	188±51[b]	0.004**
	AA	0	130 ± 0	0	---
	P-value	0.1	0.2	0.4	---
HbA1c	GG^+	10 ± 2 [a]	8 ± 2 [b]	8±2[b]	0.02*
	GA	10 ± 2	8 ±2	8±1	0.1
	AA	0	7±0	0	---
	P-value	0.7	0.4	0.6	---
Pentosidine	GG^+	662.428±214.681[a]	686.246±185.500[a]	282.063 ± 80.652[b]	0.001**
	GA	713.589±146.167[a]	689.607±193.400[a]	225.278 ± 38.509 [b]	0.001**
	AA	0	687.729± 0	0	---
	P-value	0.5	0.9	0.05*	---
CML	GG^+	2135.778±280.209[a]	2073.646±321.505[a]	1099.279 ±266.040 [b]	0.001**
	GA	2284.544±358.672[a]	2192.880±103.742[a]	921.147 ±41.561[b]	0.001**
	AA	0	1949.631±0.0	0	---
	P-value	0.2	0.6	0.06	---
GLO-1	GG^+	32.823±6.075 [a]	34.618±5.667 [a]	16.987 ± 4.108 [b]	0.001**
	GA	28.839±6.065 [a]	33.052± 6.624[a]	16.059 ± 4.976 [b]	0.001**
	AA	0	36.229± 0.0	0	---
	P-value	0.1	0.8	0.5	---
MG	GG^+	24.113 ±8.007 [a]	23.316 ±6.198 [a]	9.673 ± 2.126 [b]	0.001**
	GA	23.685 ±9.249[a]	27.386±4.831 [a]	10.070 ± 1.567 [b]	0.001**
	AA	0	41.067±0.0	0	---
	P-value	0.9	0.01*	0.6	---

Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letters are not significantly different. NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, DWR: diabetic without retinopathy, N: number, SD: standard deviation,N: number, CML: Carboxymethyl lysine, GLO-1: Glyoxalase-1, MG: Methylglyoxal, ***: a very highly significant difference(P<0.01),+: The wild genotype.

The Associations of rs6706649 SNP Polymorphisms in NFE2L2 gene with the studied parameters

Binary logistic regression analysis was used to predicate the association of rs6706649 SNP in the NFE2L2 gene and the serum levels of pentosidine, GLO-1, CML and MG. Table 8 displayed that the changing from the wild genotype (GG) to mutant (AA) and heretogynotypes (GA) had a significant positive correlation with the increase serum levels of GLO-1 and a significant negative correlation with increase serum level of MG in patients with diabetic retinopathy. However, a non-significant association between the genotype carries the mutant allele (G) and the serum levels of pentosidine, CML, GLO-1 and MG in patients without retinopathy group.

Table 8. Binary logistic regression analysis of genotypes to predict association of the rs6706649 SNP with studied biomarkers

Variables	DR		DWR	
	R.C	P- value	R.C	P-value
Pentosidine	-0.006	0.186	0.005	0.792
CML	-0.002	0.3	-0.032	0.345
GLO-1	0.348	0.015*	-0.553	0.280
MG	-0.225	0.012*	0.407	0.599

DR: diabetic retinopathy, DWR: diabetic without retinopathy, CML: Carboxymethyl lysine, GLO-1: Glyoxalase-1, MG: Methylglyoxal, R.C: regression coefficient*: significance difference (P<0.05).

Discussion

A cascade of mechanisms associated to hyperglycemia has been identified in the origin and evolution of diabetic retinopathy, which has a multifactorial onset [16]. Findings from this study indicate an association between the duration of diabetes and the occurrence of DR. A significant increase in HbA1c was observed in PDR when compared with NPDR and DWR. These results in agreement with other studies that demonstrated statistically significant increase in HbA1C levels in group with PDR compared to NPDR and DWR groups [17,18]. Glycalated hemoglobin (HbA1C) is biomarker of glycemic control in DM condition because it describes blood glucose levels in the last 60-90 days [19]. Glycemic management is crucial for diabetic microvascular problems. Microvascular problem risks decrease 37% for every 1% decrease in revised mean HbA1c [20].Under typical physiological circumstances, the formation of AGEs is limited and controlled. However, when diabetes patients experience persistent hyperglycemia, the serum levels of AGEs are significantly greater than in the normal, non-diabetic population. On the other hand, AGE production can be triggered by certain internal causes like inflammation and oxidative stress [21]. There was significant increase in the serum levels of MG, GLO-1, pentocidine and CML in DR groups as compared to DWR group. However, a non-significant difference in the above studied biomarkers between PDR and NPDR groups. This finding was convenient with other researches which observed that among the AGEs, the concentration of serum CML and pentosidine markedly increased in individuals with PDR compared to NPDR or people without DR [22,23]. According to research by Choudhuri et al., diabetic patients with NPDR and PDR had considerably higher levels of reactive oxygen species (ROS) in their peripheral blood mononuclear cells (PBMCs) than diabetic patients without DR and control subjects [24]. N-ε-CML showed a strong correlation with this increment. N-ε-CML has been suggested by several scientists as the critical molecule that initiates ROS generation, which results in lipid peroxidation and oxidative DNA damage [25].By correlating serum CML levels with retinal thickness and neurodys function ,the EUROCONDOR trial was able to detect early aberrant DR findings and monitoring its progression [26]. MG is considered the most important precursor of AGEs, resulting in the development of MG-derived AGEs. MG is produced during glycolysis and gluconeogenesis by the breakdown of many compounds. GLO1 is the rate-limiting enzyme in the glyoxalase system, which catalyzes the major detoxification step of MG [27]. Previous study demonstrated that serum MG-H1 levels are higher in type 2 diabetic patients with retinopathy than those without retinopathy which is concurrent with our findings [28].

Increased glycolytic flux combined with oxidative stress can increase the generation of MG, particularly in diabetes. Plasma levels of MG are higher in patients with diabetes than in healthy controls, and might contribute to the pathogenesis of DR [29,30]. The findings of this study about genetic polymorphism, which includes 90 type 2 diabetic patients (60 patients with retinopathy and 30 without retinopathy), revealed the presence of rs6706649 (G >A) mutation in those groups. The prevalence of GG wild genotype and GA heterozygote genotype were higher in DR group than DWR group. However, the AA mutant genotype was carried only by the DR group and

absent in the DWR group. The G and A alleles were a predominant in DR group. All of the above results were statistically non-significant. Unfortunately, there is a lack of published research on the effects of this mutation on the blood levels of the glyoxalase enzyme in type 2 diabetic patients with retinopathy. The GG variant had a higher frequency in the PDR group than in the NPDR group. The G allele was predominant in PDR group in contrast with the A allele was more prevalent in NPDR group. The rs6706649 (G >A) genotypes were not associated with the development of diabetic retinopathy in type 2 diabetic patients which is in agreement with the previous research [(7)]. NFE2LE stress-responsive transcriptional pathway modifies GLO1 activity through the ARE found in exon 1 of Glo1. The NRF2–ARE pathway regulates several genes linked to MG metabolism and defense against oxidative stress. Under oxidative stress, the basal and inducible expression of GLO1 is increased by NRF2 binding to the Glo1-ARE [(31)]. The rs6706649 (G >A) SNP of NFE2L2 gene disrupt ARE binding by lowering NFE2L2 transcriptional activity resulting decrease expression of downstream target antioxidant protein and increased severity of implicated diseases [(32)]. The results in the current study demonstrated increase oxidative stress marker which includes MG, CML and pentosidine indicate that the rs6706649 (G >A) SNP of NFE2L2 gene attenuate the NFE2L2 transcriptional activity in DR group. The DR groups carriers of A mutant allele had positive correlation with increase serum GLO-1 level and negative correlation with serum MG level. In the presence of uncontrolled hyperglycemia, the glyoxalase enzyme system becomes less efficient. The cells lose NADPH because the flux through the pentose phosphate shunt decreases. The glyoxalase system is unable to function optimally due to a decrease in GSH regeneration. Accumulation of MGO—20,000 times more reactive than glucose—to produce AGEs results from down regulation and lower effectiveness of GLO-I [(33)]. In line with the current study results, the concentrations of glyoxalase I was significantly increased in diabetic patients with retinopathy ($P < 0.001$) relative to controls [(34)]. Genetics affect the environment. Thus, polymorphisms must be assessed across different populations [(35)]. This study had a few drawbacks. One was that it only included two diabetic endocrine centers in Baghdad city. Furthermore, the measurement of NFE2L2 in vitreous fluid was not possible in this investigation. In addition, the study may not be able to accommodate a sufficient amount of time for follow-up between pre- and post-treatment groups of diabetic retinopathy patients owing to capacity constraints.

Conclusion

The A mutant allele of rs6706649 (G >A) had a significant positive correlation with the increased serum GLO-1 levels in DR group. The DR group were presented with increased serum MG, GLO-1, pentosidine and CML levels. These biomarkers can serve as prognostic indicator for diabetic retinopathy.

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Conflicts of Interest

The authors declare that there is no conflict of interest

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Ethics Statements

This study was approved by the Ethical and Scientific Committee of the College of Pharmacy at the University of Baghdad, Iraq with Ethical approval with the (REAFUBCP3112023A).

Author Contribution

All authors reviewed the results and approved the final version of the manuscript.

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تأثير التباين الجينومي لـ NFE2L2 (rs6706649) على مستويات الجلايوكسالاز-١ في عينة من المرضى العراقيين المصابين بداء السكري من النوع ٢ الذين يعانون من اعتلال الشبكية

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الخلاصة

يعد العامل النووي المرتبط بالكريات الحمر -٢ (NFE2L-2) أحد مسارات إشارة الإجهاد الداخلية المضادة للأكسدة. قد يكون تعدد أشكال النوكليوتيدات المفردة (SNP) أو المتغيرات الجينية لـ NFE2L-2 مسؤولاً عن نشأة اعتلال الشبكية السكري. الجلايوكسالاز-١ هو الإنزيم الذي يحد من معدل إزالة السموم من ميثيل جليوكسال إلى D-لاكتات. يتم تنظيم نشاط GLO1 بواسطة عامل النسخ NFE2L2. الهدف من هذه الدراسة هو التأكد مما إذا كان هناك ارتباط بين متغير الجين rs6706649 من NFE2L2 ومستويات GLO-1 في الدم في مجموعة من مرضى السكري العراقيين الذين تم تشخيص إصابتهم باعتلال الشبكية السكري . شملت هذه الدراسة تسعين مريضاً تم تشخيص إصابتهم بداء السكري من النوع الثاني (٤٨ أنثى و٤٢ ذكراً)، وتتراوح أعمارهم بين ٤٠ إلى ٨٠ عاماً. تم تقسيم المشاركين إلى المجموعتين التاليتين: المجموعة أ؛ تم تشخيص ٦٠ مريضًا مصابًا بداء السكري من النوع ٢ باعتلال الشبكية السكري (من بينهم ٢٩ مريضًا يعانون من اعتلال الشبكية السكري غير التكاثري (NPDR) و٣١ مريضًا يعانون من اعتلال الشبكية السكري التكاثري (PDR)) والمجموعة ب: ٣٠ مريضًا بدون دليل على اعتلال الشبكية السكري (DWR).فرق غير مهم في التنميط الجيني وترددات نقل الأليل لـ SNP (G/A) rs6706649 بين مجموعات DR وDWR. كما كان هناك فرق غير معنوي بين مجموعات مرضى PDR و NPDR فيما يتعلق بالأنماط الجينية والأليلات المختلفة لـ (G/A) rs6706649 لجين NFE2L2. ومع ذلك، كان للتغير من النمط الوراثي البري (GG) إلى الطفرات (AA) والأنماط الوراثية (GA) علاقة إيجابية معنوية مع زيادة مستويات مصل GLO-1 وارتباط سلبي كبير مع مستوى مصل ميثيل جليوكسال (MG) في المرضى الذين يعانون من مرض السكري. اعتلال الشبكية. علاوة على ذلك، كانت مستويات البنتوسيدين وكربوكسي ميثيل ليسين (CML) وGLO-1 وMG أعلى بكثير في DR بالمقارنة مع مجموعات DWR. كان للأليل A المتحول لـ (G> A) rs6706649 علاقة إيجابية كبيرة مع زيادة مستويات GLO-1 في الدم في مجموعة DR. وفي الوقت نفسه، زادت مجموعة DR مستويات MG، GLO-1، البنتوسيدين وCML في الدم. وبالتالي، يمكن أن تكون هذه المؤشرات الحيوية بمثابة مؤشرات إنذار لاعتلال الشبكية السكري.

الكلمات المفتاحية: الإجهاد التأكسدي، تعدد الأشكال الجيني NFE2L-2، GLO-1، MG، اعتلال الشبكية السكري